



# High-throughput, unbiased analysis of dose-dependent antiproliferative drug effects

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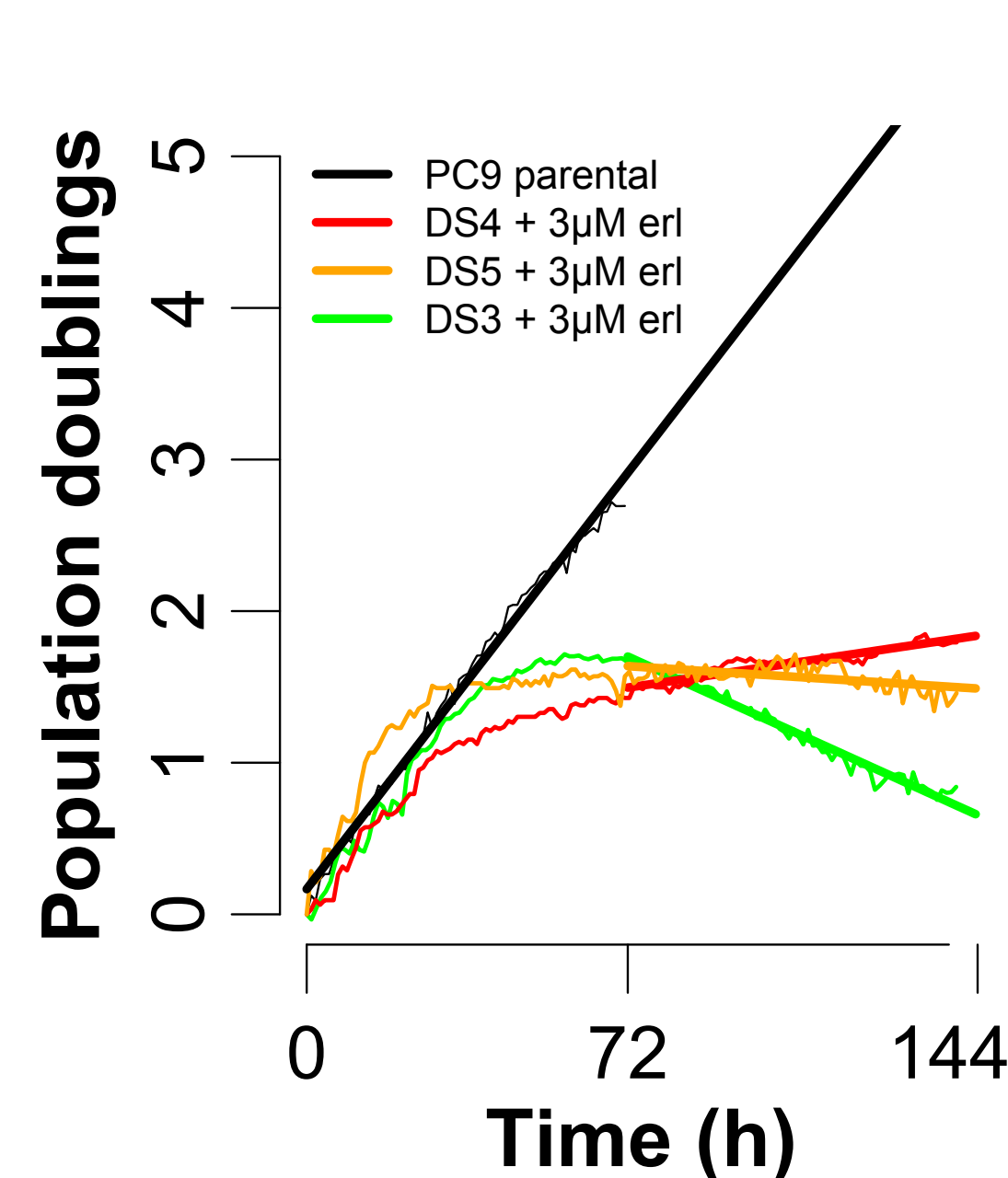
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## SUMMARY

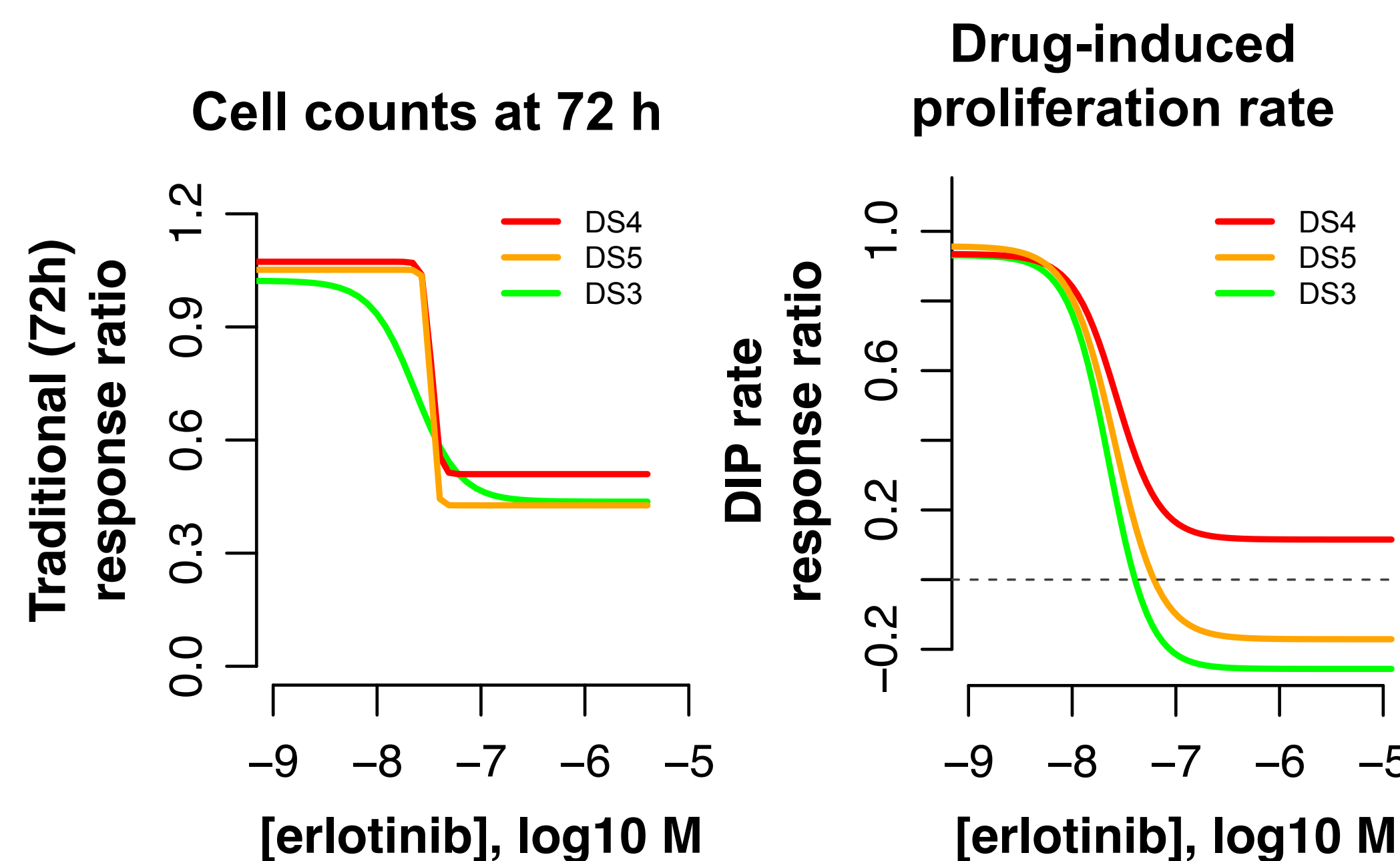
Evaluating antiproliferative drug activity on cells in vitro is a widespread practice in basic biomedical research and drug discovery. Typically, quantitative assessment relies on constructing dose–response curves from end-point assays, for which the de facto standard metric is the number of viable cells 72 h after drug addition. Using theoretical modeling and experimentation, we have shown that current metrics of antiproliferative small molecule effect suffer from time-dependent bias, leading to inaccurate assessments of parameters such as drug potency and efficacy. We proposed the drug-induced proliferation (DIP) rate, the slope of the line on a plot of cell population doublings versus time, as an alternative, time-independent metric that eliminates bias due to proliferation rates and drug activity delays. The DIP rate analytical platform incorporates time-lapse cell imaging, automated image processing and cell counting. Feature acquisition (e.g., division or death) at the single-cell level is also available, if desired. These data can be incorporated into mathematical models to predict cellular response dynamics to drug treatment, as well as both genetic and non-genetic perturbations. Here we present our approach for scaling the DIP rate platform to a high-throughput screening format capable of obtaining data for approximately 13,000 unique conditions at 12 time points over 5 days. With appropriate, measurable conversion coefficients, it should be possible to translate this information to in vivo experiments and other applications.

## CELL POPULATION DYNAMICS AFFECT DOSE–RESPONSE CURVE PARAMETERS



Three randomly selected single-cell clones of the PC9 lung adenocarcinoma cell line harboring mutant EGFR exhibit distinct kinetics in response to EGFR inhibition with erlotinib (left). Note that due to the delayed effect of the drug, all sublines appear similarly responsive to drug at 72 h but show divergent behaviors thereafter.

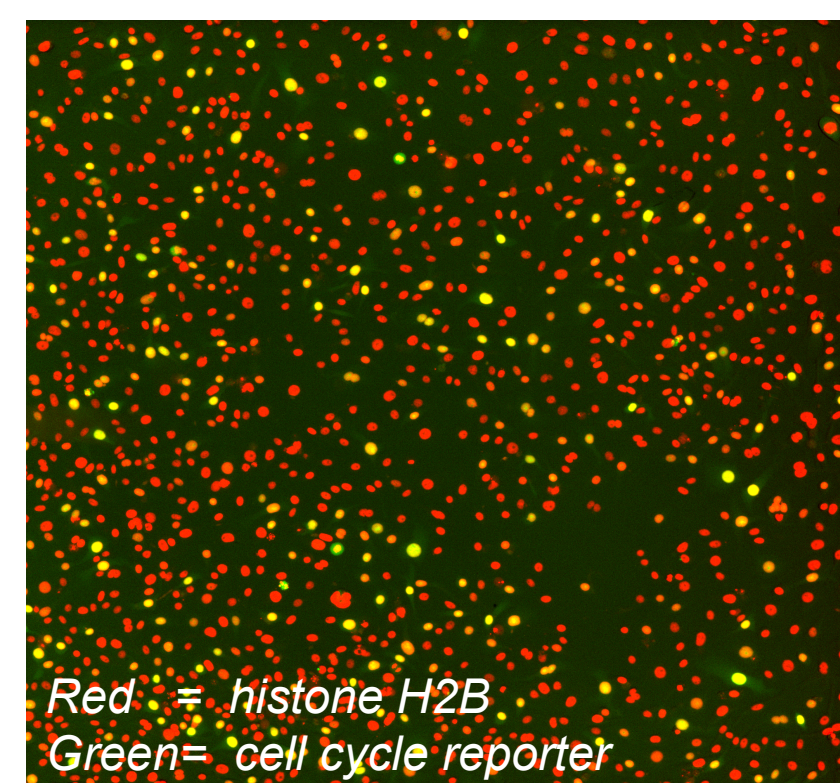
How drug effect is measured significantly affects the resultant dose–response curves (below).



**NOTE: All three sublines have essentially the same EC<sub>50</sub> (~ 30 nM) but have very different growth kinetics when [erlotinib] > 100 nM**

## DATA ACQUISITION

Cells are engineered to express fluorescent protein-conjugated histone H2B and a cell cycle indicator (mAG-gem1-110) as described (Tyson et al., Nat Methods 2012, doi:10.1038/nmeth.2138). Cells are seeded into 384-well plates at densities ranging from 200–800 cells per well. Up to 42 plates can be run in a single experiment in the HTS core and each plate is uniquely identified using barcodes. Plates are loaded into a Cytomat cell incubator and transported one at a time to the barcode reader and into an ImageXpress Micro XL bioimager (Molecular Devices). Images are acquired in each well (308 wells per plate) continually over 72 h when medium and drug are replaced followed by another 48 h of imaging. A video of the automated image acquisition can be seen by scanning the QR codes below on your smartphone or tablet.



Scan code for video of automated image acquisition (robotic plate handling)



Scan code for video of example time-series image stack: PC9 cells + 1 nM osimertinib; nuclei only; grayscale

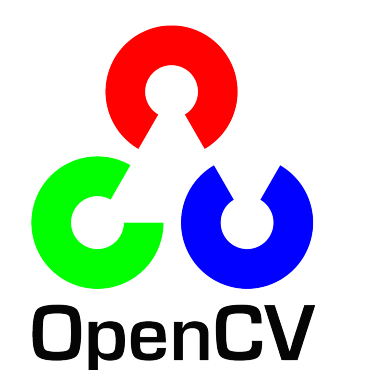
## HIGH-THROUGHPUT IMAGE PROCESSING

To accommodate the very large datasets (over 200,000 images and >2 TB per experiment), a parallel-processing-based image analysis pipeline was developed. We used freely available software tools and custom-written code in R and Python and Python-based tools for distributed processing (RabbitMQ and Celery)

**Assemble file information** (plate ID, well name, fluorescent channel, image name, exported from ImageXpress in HTS core)



**Organize file info and other parameters for Python/OpenCV image processing into many thousands of unique “tasks”**



**RabbitMQ™**  
Send “task” list to RabbitMQ for distribution



**Celery “workers” running on separate processing units pull tasks from the task queue and generate separate output files**



**one experiment results in >200,000 image processing tasks**

**32 cores simultaneously processing tasks require ~16 h**

## DATA VISUALIZATION AND ANALYSIS



**Thunor**

A web-based data management and visualization tool

Example dataset: 6 BrCa cell lines, 36 drugs, 10 concentrations each drug, technical duplicates, images acquired every ~2h for 120h (>200,000 measurements). Cell count data are uploaded into the Thunor web repository and combined with other data into the DIP rate database.

As of Feb, 2018, the DIP rate database contains data from **over 30 cell lines and over 150 drugs**

Cell lines enriched in EGFRmut LuCa, BRAFmut melanoma, and triple-negative BrCa

Drugs include the FDA-approved oncology panel (126 drugs)

